Predicting Natural Neuroprotection in Marine Mammals: Environmental and Biological Factors Affecting the Vulnerability to Acoustically Mediated Tissue Trauma in Marine Species

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LONG-TERM GOALS

The primary goal of these studies is to investigate the relative vulnerability of marine mammals to acoustically mediated trauma from emboli formation. By evaluating key environmental, behavioral and physiological factors involved in the movement of gases at the whole animal and tissue levels we intend to identify factors contributing to lipid, nitrogen, and carbon dioxide gas mobilization, and concomitant tissue damage at depth. The results of this project will enable the development of environmentally sensitive schedules for oceanic acoustic activities by identifying those species most susceptible to tissue injury.

OBJECTIVES

To accomplish these goals we are focusing on three key questions:

1. **Environmental:** ***Does elevated environmental temperature compromise the dive response that safeguards marine mammals from decompression illness?*** This is being tested by measuring cardiovascular and metabolic parameters of trained bottlenose dolphins during sedentary and active periods while diving in warm and cold water.

2. **Behavioral:** ***Do increased levels of neuroprotecting globins in the brain correspond to increased plasticity of the dive response during voluntary activity by marine mammals?*** Here we evaluate the physiological significance of elevated globin levels that we have discovered in the cerebral cortex of marine mammals. This is being tested by comparing behaviorally induced variability in the dive response (as manifested by changes in the level of bradycardia and peripheral circulation) in deep and shallow diving mammal species including bottlenose dolphins and Weddell seals.

3. **Physiological:** ***Does globin deposition and coincident neuroprotection of the cerebral cortex change with developmental stage in marine mammals?*** By evaluating globin deposition profiles

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from carcasses ranging in age from neonates to adults, we are investigating how age influences neuroprotective mechanisms in a wide variety of marine mammal species.

Together these studies will enable us to determine why some marine mammal species, such as the family of beaked whales, appear more susceptible to non-auditory tissue damage as may occur in conjunction with navy and oil exploration sound operations. We take into account several recent hypotheses regarding emboli formation, observed behavioral responses of marine mammals to low- and mid- frequency sound production, as well as the results of our studies to develop predictive models for susceptibility to decompression illness.

APPROACH

This study uses two approaches to determine the relative susceptibility of different marine mammal species to acoustically mediated trauma, 1) molecular and biochemical evaluation of neuroprotection at the tissue level, and 2) whole animal/physiological assessments to determine the impact of behavioral and environmental challenges to the dive response. Because stranded marine mammals often display behaviors associated with neural dysfunction (i.e. disorientation, poor localization and righting responses), and neural tissues are exceptionally vulnerable to decompression damage, we focus on the central nervous system and its relationship to the dive response.

Laboratory studies at the tissue level are assessing the presence and function of oxygen binding circulating (hemoglobin) and resident (cytoglobin and neuroglobin) globin proteins in the brain, and myoglobin in locomotory muscles. Recently, a survey of shallow and deep diving species enabled us to determine the effects of routine dive capacity on the expression of these globins (Williams *et al.,* 2008). Our current studies build on this foundation to evaluate how these different globins affect the vulnerability of a variety of marine mammal species to hypoxia associated with decompression syndromes. We are also examining how age influences globin deposition and coincident avoidance of tissue damage from hypoxia in immature and mature marine mammals. Ultimately, this will allow us to determine if specific segments of marine mammal populations are more susceptible than others to tissue damage.

Team members include specialists in morphology and pathology of marine mammals (M. Miller, CA Dept. Fish and Game; D.A. Pabst, Univ. North Carolina-Wilmington), globin chemists (D. Kliger and R. Goldbeck, UCSC), molecular biologists (M. Zavanelli, UCSC) and physiologists (T.M. Williams, D. Casper, N. Thometz and S. Noren, UCSC.)

The second component of this study examines the susceptibility of marine mammals to decompression illness at the whole animal/physiological level by monitoring behaviorally induced variability in the dive response. Because nitrogen transfer and decompression illness are linked to tissue perfusion, relaxation of the dive response in marine mammals has the potential to increase susceptibility to neural tissue damage either by preventing the removal of nitrogen or altering the perfused tissue pool available for nitrogen dispersal. The effects of two physiological mechanisms known to alter blood flow are being investigated, exercise and heat. In the first series of tests we are evaluating the effects of exercise intensity on changes in the dive response of bottlenose dolphins. Dolphins are trained to dive and exercise at varying depths. Variability in bradycardia and peripheral vasoconstriction are subsequently monitored as the animals perform sedentary to high intensity exercise tasks. Our most

recent work provides a comparative dimension by conducting similar tests on a deep diving species, the beluga whale, and a free-ranging deep diver, the Weddell seal. A second set of tests uses this protocol to determine the effects of acute and chronic increases in environmental temperature on variability of cardiovascular responses in diving and swimming bottlenose dolphins.

Team members for this part of the program include physiologists (T.M. Williams, S. Noren, and L. Yeates from UCSC; R. Davis, Texas A&M University) and animal behaviorists (T. Kendall and B. Richter, UCSC; P. Berry, EPCOT)

WORK COMPLETED

Tissue Globin Analyses. Our team has successfully developed two assays for brain globins, a spectrophotometric test that provides total globin concentration and an mRNA expression test for relative cytoglobin and neuroglobin levels. Previously, we have used these assays to detect the presence and concentration of globins in the cerebral cortex of 16 species of mammals. This includes five species of terrestrial mammal ranging in body mass from 0.1 kg to 100 kg, and 11 species of marine mammal ranging in mass from 30 to 300 kg. Among the marine species, we have examined both coastal and pelagic divers among the small cetaceans, pinnipeds and sea otters. All have demonstrated the presence of brain globins, although the concentration varies among the various species. This year we were fortunate to obtain brain samples representing different ages from several marine mammal species including sea otters, pinnipeds and cetaceans, as well as from several subspecies of beaked whale from both the Atlantic and Pacific Oceans. Analysis of these tissues is ongoing.

Variation in Diving Bradycardia and Vascular Control during Diving. The second component of this study examines variability in the dive response of marine mammals due to exercise and environmental temperature. A major challenge was developing heart signal instrumentation that could withstand the rapid swimming movements of dolphins. We have successfully tested and collected data using a new submersible electrocardiograph/accelerometer monitor by UFI, Inc. (Morro Bay, CA). To date eight dolphins, two beluga whales, four Weddell seals and two sea otters have been examined. Heart rate during surface and submerged resting periods were collected for all four species, including an evaluation of the effects of body position on bradycardia in dolphins. During the past two years we deployed our instrument on free-ranging Weddell seals to provide comparative data for a deep-diving pinniped during foraging events. Tests with other species are ongoing.

Efforts this year have been on developing new models of dive responses for marine mammals taking into consideration the variation in bradycardia that occurs with different levels of activity. Specifically we are relating variation in the dive response and the incidence of cardiac arrhythmias to the level of exercise performance and dive depth. We have completed a series of exercise tests for dolphins freely-diving to 3 m, 10 m and 20 m and Weddell seals diving to >400 m. We have also collected data on the combined effects of exercise and increased environmental temperature on cardiovascular responses in dolphins using heat flow and changes in core body temperature as metrics for alterations in blood flow. Data analysis for these species is ongoing and includes cardiovascular signatures for different levels of exercise intensity and water temperature. Comparative studies on beluga whales and sea otters are scheduled to continue this year. The results from the dolphin tests have been presented at the **Society for Integrative and Comparative Biology** meeting (Seattle WA, January 2010). Predictive models of

gas movement in the cardiovascular system, aerobic dive limits, and susceptibility to decompression illness based on our results were discussed at the **Diving Marine Mammal Gas Kinetics Workshop** (Woods Hole MA, April 2010) and were incorporated into three manuscripts that were published this year. The results for the species examined were presented in a series of talks and posters at the **Society of Marine Mammalogy** meeting (Tampa FL, November 2011) and at the **Society for Integrative and Comparative Biology** meeting (Charleston SC, January 2012).

RESULTS

The major research effort this year has been on a detailed analysis of ECG recordings from actively diving marine mammals in both controlled experiments and free-ranging conditions. We have discovered that in contrast to the common description of diving "reflexes" in marine mammals, cardiovascular responses during diving are highly variable in the species examined (Davis and Williams 2012; Noren *et al*., 2012). Both dolphins and Weddell seals demonstrate a release from bradycardia with increased locomotory effort that is modified by exercise intensity and behavior. Dolphins showed a 2.2-fold increase in heart rate from submerged rest to 72 strokes.min^{-1} at preferred swimming speeds; seals showed a 3.8-fold change in heart rate across speeds.

Importantly, this detailed analysis has revealed the conflicting autonomic signals received by the marine mammal heart during submerged activity (Fig. 1). These include sympathetic innervation of the atria and ventricles which increases heart rate with exercise, and parasympathetic vagal tone at the level of the sino-atrial node which mediates suppression of heart rate in the diving marine mammal.

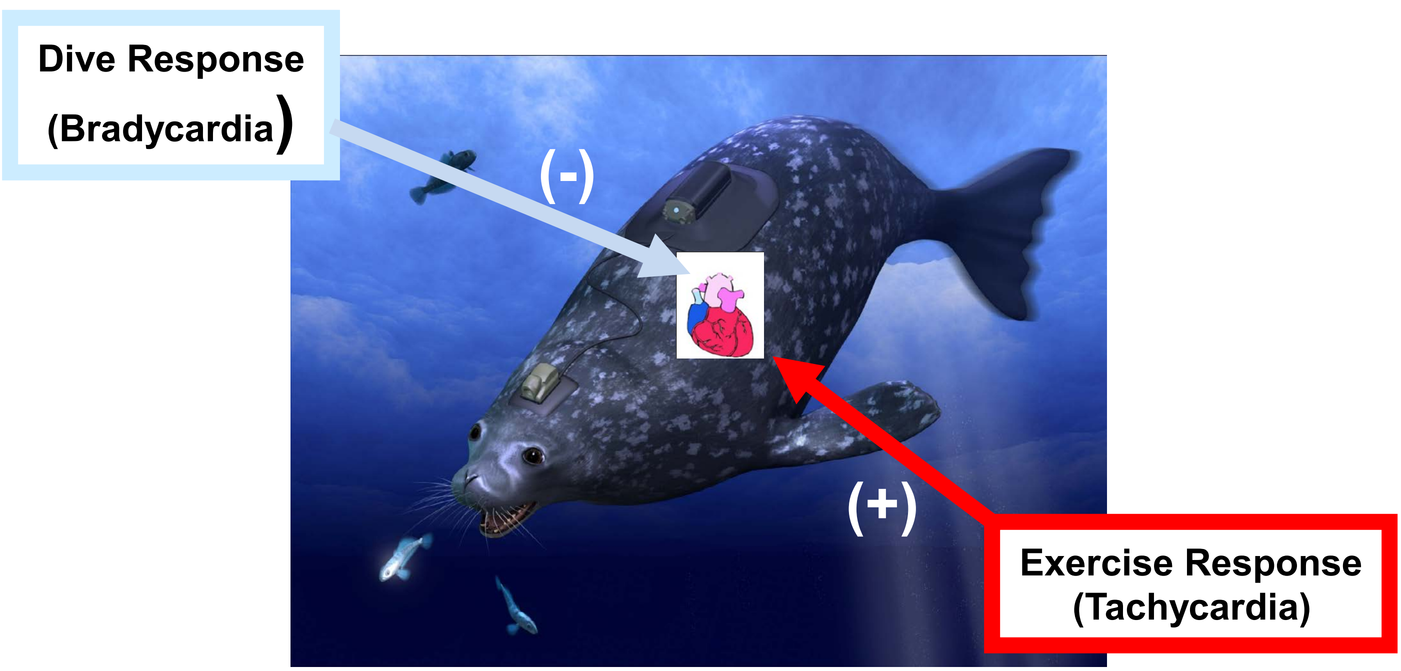

Figure 1. Conflicting autonomic nervous signals controlling heart rate in diving marine mammals. Under free-ranging conditions suppression of heart rate due to the dive response (blue box) is superimposed on elevation in heart rate associated with exercise responses for locomotion (red box).

Rather than graded transitions between tachycardia when on the water surface and bradycardia when submerged, control of the heart oscillates between these states when the animal dives (Fig 2). Cardiac arrhythmias including ectopic beats and pre-ventricular contractions have been observed at the transition points between bradycardia and tachycardia as divers ascend. The depth of occurrence for cardiac arrhythmias appears to be species-specific. Further studies to determine the instigating factors promoting these arrhythmias are planned.

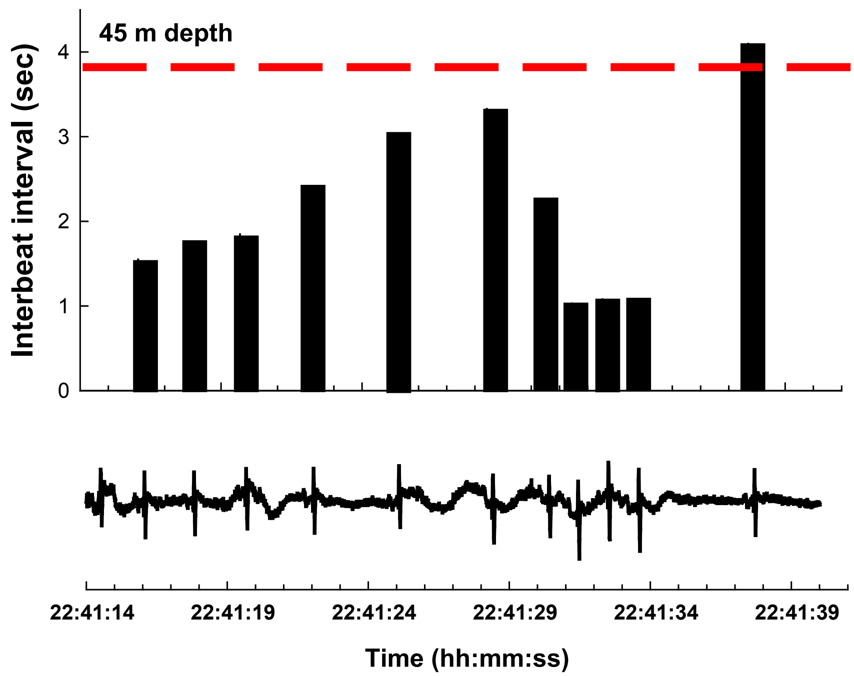

Figure 2. Cardiac instability in a deep-diving marine mammal. The bottom portion of the figure shows a typical ECG trace for a diving Weddell seal ascending from a 300 m dive. The corresponding changes in inter-beat interval (IBI) during the ascent as the seal passes 45 m (upper black bars) demonstrates marked cardiac variability compared to the stable IBI during diving at maximum depth (red dashed line). (From Williams et al., in prep.)

These studies indicate a heretofore unknown level of cardiovascular variability and autonomic conflict during diving in marine mammals. This has marked implications for physiological stability and the mobilization of gases during submergence, especially during prolonged, high speed or deep dives. Our data indicate that specific behaviors or segments of a dive are riskier than others in terms of inducing cardiovascular instability. From our free-ranging studies we find that marine mammals tend to maintain preferred levels of exercise which result in only modest changes in the dive response. This not only shapes preferred foraging tactics, but likely contributes to the exceptional sensitivity of some marine mammal species (e.g. deep diving beaked whales) to anthropogenic-derived acoustic disturbances through altered locomotory behaviors including increased swimming speed, ascent rate, stroke frequency).

IMPACT/APPLICATIONS

Our recent findings on variability in the cardiovascular response to diving and in tissue globin levels provide:

1. A new perspective on physiological stability in deep diving mammals. By examining a wide variety of mammalian species living in different habitats, we demonstrate how malleable the cardiac response of mammals is regardless of adaptations for diving. The marine mammal heart shows levels of instability that is surprisingly similar to that of humans during accidental cold water submersion. This information is now being used by our team and others to create new models of gas mobilization that will define species-specific robustness or vulnerability to anthropogenic disturbance.

2. An assessment of the importance of globin proteins. Since neuroglobin and cytoglobin have been associated with neuronal survival following stroke and other ischemic insults with cardiovascular accidents, the results are relevant to many of the leading causes of mortality in the United States. Differences in resident neuroglobins may help to explain the relative susceptibility of deeper diving species to barotrauma following exposure to anthropogenic noise.

3. New techniques for clinical, ecological, behavioral and physiological studies. The instrumentation developed for monitoring cardiovascular changes in freely-diving marine mammals and the predictive models being tested provide new tools for assessing the response of wild mammals to anthropogenic disturbance. In addition, our study is developing new biochemical methods and animal models for the assessment of brain globins that should be of interest to a wide variety of comparative and medical neurophysiologists.

RELATED PROJECTS

None.

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